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Original research article

ANTIMICROBIAL RESISTANCE PROFILE AND CHARACTERISATION OF ENTEROCOCCUS SPECIES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: *Enterococcus* is one of the leading causes of nosocomial infections, with *E faecalis* and *E faecium* accounting up to 90-95% of clinical isolates. During recent years, the occurrence of other *Enterococcal* species from clinical samples increased with the properties of resistance to many antibiotics. Thus appropriate identification of *Enterococci* at species level is crucial for the management and prevention of these bacteria in hospital settings. Hence, this study was undertaken to highlight the incidence of multi drug resistant enterococcal species from various samples from human infections, in a tertiary care hospital. **Methods:** This work was conducted in our institution from January 2009 to December 2011. About 112 *enterococcal* isolates from various clinical specimens were included in the study. The isolates were identified by standard microbiological methods. Antimicrobial susceptibility testing was carried out by using Kirby-Bauer disc diffusion method. The prevalence of High level Gentamicin resistance was identified. Vancomycin resistance was assessed by E-test. **Result:** The commonest species identified was *E faecalis* (87.5%), followed by *E faecium* (8.9%). 14% of isolates produced beta haemolysis and gelatinase. 15% and 24% were the haemolytic and gelatinase producing *enterococci*. High level resistance was shown towards tetracycline, Amikacin, Cholramphenicol. Vancomycin resistance was identified in single isolate. **Conclusion:** There is achange in isolation pattern of *enterococcal* species. Besides, there is an increased rate of infection with multidrug resistant *enterococci* species, which necessitates frequent antimicrobial surveillance.

Keywords: Enterococcus, E.faecalis, Antimicrobial resistance pattern, High level Gentamicin resistance

INTRODUCTION

In recent decades, most of the pathogenic bacteria developed resistance to one or more antimicrobial agents. *Enterococci* are commensals ~~ism~~ of the gastrointestinal tract of human beings. They have gained more clinical importance due to their multidrug resistance[1, 2]. The ability of *Enterococci* to colonize the gastrointestinal tract of hospitalized patients for long periods is a crucial factor that influences the development of drug resistance[3]. The CDC in a

survey indicated that a high percentage of hospital acquired infections are caused by *Enterococcus* next to MRSA and ESBL producers[4]. Infection with Vancomycin resistant *Enterococci* is associated with increased mortality, length of hospital stay, admission to the ICU, surgical procedures & cost[5]. The common species which causes infection are *E. Faecalis* (80-90%) and *E faecium* (5-10%)[6]. Recently there is an increase in isolation rate of *E faecium* & other species from various clinical samples [4, 7].This study aimed to determine the prevalence of multi drug resistant *Enterococcus* from various clinical specimens and changing trends in isolation along with their virulence characterisation.

MATERIALS AND METHODS

This study was conducted in the department of microbiology, Melmaruvathur Adhiparasakthi Institute of Medical Sciences and Research, Tamil Nadu, India from January 2009 to December 2011. The samples were collected from both outpatients and inpatients of all age groups of both genders. *Enterococcal* species isolated from urine, blood, pus, sterile body fluids and aspirates were included in the study. A total of 112 *Enterococcal* isolates were included in the study.

Identification of *Enterococcus* was done using the following parameters (i) Colony morphology on blood agar, Cystine Lactose Electrolyte Deficient agar and Mac Conkey agar (ii) Gram's stain (iii) Catalase (iv) Bile Esculin (v) Heat resistance (vi) Salt tolerance. Subsequently, speciation was performed by sugar fermentation, pyruvate fermentation, motility and reduction of tellurite in tellurite blood agar plate[8].Determination of virulence factors like haemolysis and gelatinase were carried out by appropriate tests [9].

Antibiotic susceptibility pattern

Antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion method using the following antibiotic discs: Vancomycin (30µg), Erythromycin (15µg), Amoxycillin (10µg), Ofloxacin (5 µg), Amikacin (30 µg), High Level Gentamicin (120 µg), Ciprofloxacin (5 µg), Chloramphenicol (30 µg), Tetracycline (30 µg). *E.faecalis* ATCC 29212 was used as a control strain for disc diffusion tests[10].

RESULTS

Out of 112 Enterococcal isolates consists of 98 *E fecalis* (87.5%), 10 *E faecium* (8.9%), 3 *E durans* (2.6%) and 1 *E raffinosus* (0.89%). The maximum number of *Enterococcus* was isolated from urine 98/112 (87.5%), followed by blood 6/112(5.3%) (Table-1).The baseline data of patients infected with *Enterococcus* were given in Table -2. Sixty four *Enterococcus* species had been isolated as a mixture and their pattern of isolation was described in Table-3.

Fifty eight (51.7%) isolates were gamma haemolytic and 31 (27.6%) 37 (33 %) were β and alpha haemolytic Enterococci respectively. Description of virulence characteristics has been shown in Table-4.

E faecalis was resistant to tetracycline (67%), Chloramphenicol (63%) and Amikacin (60%). *E faecium* showed high level resistance to Ciprofloxacin (100%), Erythromycin (80%), Amikacin (60%). *E durans* was resistant to Ciprofloxacin (100%), Erythromycin (66%), Amoxycillin (66%), tetracycline (66%) & tetracycline (66%). *Enterococcus* showed maximum sensitivity for Vancomycin (99%), amoxicillin (65%), Ofloxacin (58%).

Table .1: Specimen wise distribution of Enterococcus

Specimens	E faecalis (98)	E faecium (10)	E durans (3)	Eraffinosus (1)
Urine (98)	90	7	-	1
Blood(6)	5	1	-	-
Pus(3)	1	2	-	-
Tracheal aspirate (1)	1	-	-	-
Ascitic fluid (1)	-	-	1	-

Table.2: Basic data of patients with Enterococcus infection

Variable		No. of Enterococcal isolates	Percentage
Sex	Male	83	74
	Female	29	26
OP/IP	Inpatient	64	57
	Outpatient	48	43
Age	<20 years	11	10
	20-40	56	50
	40-60	23	20.5
	60-80	22	19.6

Table.3: Pattern of Enterococcus isolation

Isolate	No. of isolates	Percentage
Enterococcus	60	53.5
Enterococcus+E. coli	14	12.5
Enterococcus+Klebsiella species	10	8.9
Enterococcus+Candida species	9	8.0
Enterococcus+ Staphylococcus aureus	7	6.2
Enterococcus+ CONS	7	6.2
Enterococcus+ CONS+ Candida species	5	4.4

Table.4: Virulence characteristics of Enterococcus

Virulence factor	No. Of isolates	Percentage
Beta haemolysis	17	15.15
Gelatinase production	27	24
Both	14	12.5

Table-5 Antimicrobial susceptibility pattern of Enterococcus

Drug	E faecalis (98)		E faecium (10)		E durans (3)		E raffinosus (1)	
	S	R	S	R	S	R	S	R
Erythromycin	51	47	2	8	1	2	1	0
Amoxycillin	77	21	6	4	1	2	0	1
Ciprofloxacin	53	45	0	10	0	3	1	0
Ofloxacin	57	41	6	4	1	2	1	0
Tetracylin	32	66	8	2	1	2	1	0
Chloramphenicol	36	62	8	2	3	0	1	0
Amikacin	39	59	4	6	2	1	1	0
High level gentamicin	68	30	8	2	3	0	1	0
Vancomycin	98	0	9	1	3	0	1	0

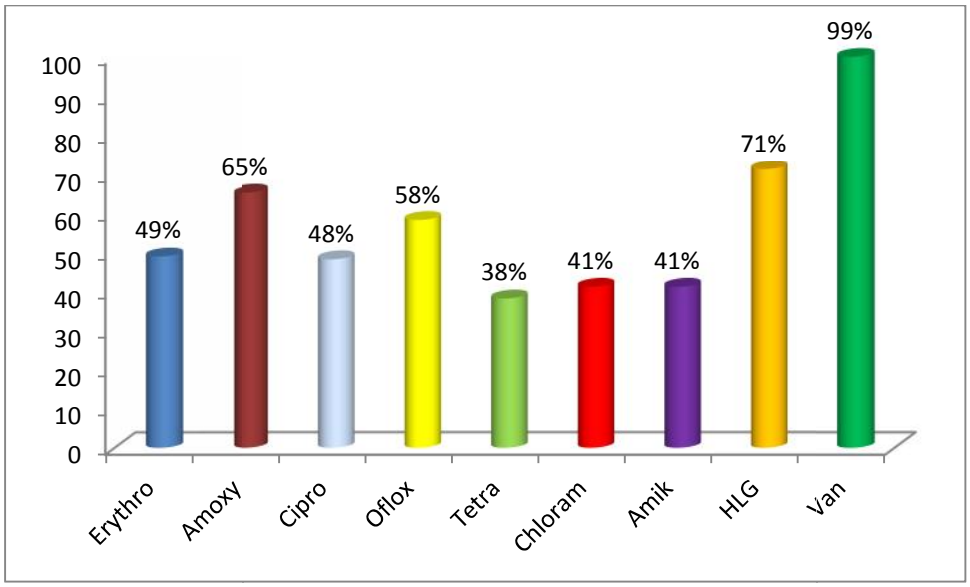

Fig.1: Sensitivity pattern of Enterococcus species

DISCUSSION

The *Enterococcus* species have now emerged as important nosocomial pathogens. Hence, it is important to know the changing patterns of the *Enterococcus* infections and the antimicrobial susceptibility pattern of isolates. In our study, about 112 *Enterococcal* isolates were recovered from various specimens. The maximum number of isolates was obtained from urine followed by blood. In some studies, pus isolates were high compared to isolates from urine[11-13].

Enterococcal infections were common in males (74%) than in females (26%). About 10% of isolates were recovered from patients below 20 years of age, of which 3 (27%) were obtained from neonates. Although the recent studies stated there is an increase in isolation of *E faecium* and other *enterococcal* species[14]. In our study, *E fecalis* (87.5%) constitute the major isolate, followed by *E faecium*(8.9%), *E durans* 2.6 %[15] and similar findings were shown by Facklam et

al study[16-18].About 53.5% of *Enterococcus* was isolated in pure culture. The remaining 46.5% were recovered with other organisms as mixture, commonly associated with *E.coli* (12.5%), *Klebsiella* (9%) and *Candida* (8%).

About 12.5% of isolates produced gelatinase and haemolysin. Seventeen (15%) and twenty seven (24%) isolates were positive only for beta haemolysis and gelatinase respectively.

E faecium and *E durans* showed 100% resistance to Ciprofloxacin, one of the commonest antibiotic used to treat urinary tract infection. HLGR was observed in 30.6% of isolates, which partially correlates with finding by studies[19]. In some studies 66% HLAR were observed[20]. About 59% of *E.fecalis* was found to be resistant to one of the commonly used antibiotic Amikacin. Twenty percentages of isolates showed intermediate sensitive to vancomycin by Kirby-Bauer disc diffusion method. All became sensitive to Vancomycin by E-test strip except one.

CONCLUSION

There is increased frequency of isolation of uncommon *Enterococcal* species. Thus, definite identification of *Enterococci* at species level is mandatory to assess their variable sensitivity pattern and treat accordingly. Detection of beta haemolysis can be taken as an additional virulence marker in routine laboratory testing. Since nearly half of the *Enterococcal* isolates were identified as a mixed bacterial growth, ultimate care should be taken before choosing empirical antibiotic therapy.

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